Endotoxin in inner-city homes: Associations with wheeze and eczema in early childhood

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Background: An inverse association between domestic exposure to endotoxin and atopy in childhood has been observed. The relevance of this aspect of the hygiene hypothesis to US inner-city communities that have disproportionately high asthma prevalence has not been determined.

Objectives: To measure endotoxin in the dust from inner-city homes, evaluate associations between endotoxin and housing/lifestyle characteristics, and determine whether endotoxin exposure predicted wheeze, allergic rhinitis, and eczema over the first 3 years of life.

Methods: As part of an ongoing prospective birth cohort study, children of Dominican and African-American mothers living in New York City underwent repeated questionnaire measures. Dust samples collected from bedroom floors at age 12 or 36 months were assayed for endotoxin.

Results: Among the samples collected from 301 participants' homes, the geometric mean endotoxin concentration (95% CI) was 75.9 EU/mg (66-87), and load was 3892 EU/m^2 (3351-4522). Lower endotoxin concentrations were associated with wet mop cleaning and certain neighborhoods. Endotoxin concentration correlated weakly with cockroach (Bla g 2: $r = 0.22$, $P < .001$) and mouse (mouse urinary protein: $r = 0.28$; $P < .001$) allergens in the dust. Children in homes with higher endotoxin concentration were less likely to have eczema at age 1 year (odds ratio, 0.70 [0.53-0.93]) and more likely to wheeze at age 2 years (odds ratio, 1.34 [1.01-1.78]). These associations were stronger among children with a maternal history of asthma.

Conclusion: Endotoxin levels in this inner-city community are similar to those in nonfarm homes elsewhere. In this community, domestic endotoxin exposure was inversely associated with eczema at age 1 year, but positively associated with wheeze at age 2 years.

Clinical implications: Endotoxin exposure in the inner-city community may be related to wheeze in the early life; however, given the inverse association seen with eczema, the long-term development of allergic disease is still in question. (J Allergy Clin Immunol 2006;117:1082-9.)

Key words: *Endotoxin, asthma, allergy, hygiene hypothesis, wheeze, inner-city, eczema*

The hygiene hypothesis asserts that the increase in the prevalence of allergic disease documented in Western society in the 20th century can be attributed, at least in part, to lifestyle changes that have led to cleaner living conditions and reduced exposure to viruses and bacteria in the first years of life.[1,2] Inverse associations between bacterial endotoxin in house dust and atopic disease have been observed in cross-sectional studies.[3,4] Endotoxin, a LPS found in the outer membrane of Gram-negative bacteria, can downregulate T_H2 cytokine production and class switching to IgE isotype in mouse models.[5] Hence, several authors have postulated that ambient exposure to high amounts of endotoxin in the home or farm environment could modulate the risk for atopy by suppressing molecular pathways responsible for IgE-mediated atopic disease.[6-8]

It has been suggested that the hygiene hypothesis may explain intercommunity differences reported in the prevalence of atopic asthma.[9] The prevalence of asthma generally is greater among industrialized countries, where living conditions appear to be cleaner and fewer bacterial infections seem to occur, than among developing nations.[10,11] However, it is unclear where inner-city homes in the United States, generally afflicted by a disproportionately high prevalence of asthma, fall in the spectrum of environmental endotoxin exposure.[4,12] One unanswered question is whether a greater level of hygiene associated with an inner-city lifestyle can explain the higher asthma prevalence

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Abbreviations used:
EU: Endotoxin unit
GEE: Generalized estimating equation
GM: Geometric mean
MUP: Mouse urinary protein
NYC: New York City
OR: Odds ratio

relative to that observed in rural and suburban areas.[12,13] Another is the extent to which endotoxin exposure modulates the risk for developing atopic disease or wheeze in this community during childhood.

Our study examined endotoxin exposure and asthma-related health outcomes in the first 3 years of life in a birth cohort of children living in New York City (NYC) neighborhoods that have reported a disproportionately high asthma prevalence.[12] We hypothesized that differences in endotoxin exposure would be associated with asthma-like symptoms, allergic rhinitis, and eczema. Our strategy was to (1) measure endotoxin in the dust from homes, (2) determine whether allergen levels, housing, and/or lifestyle characteristics were associated with endotoxin exposure in the inner city, and (3) determine whether endotoxin exposure in this inner-city birth cohort predicted wheeze, allergic rhinitis, and eczema over the first 3 years of life.

METHODS

Study cohort

Participants included 301 children recruited as part of an ongoing birth cohort, described previously, conducted by Columbia's Center for Children's Environmental Health.[14-17] Nonsmoking mothers not selected on allergic/asthmatic status of African-American and Dominican ethnicity living in Northern Manhattan and the South Bronx were recruited during the second or third trimester of pregnancy from prenatal clinics. This study was approved by Columbia University's Institutional Review Board.

Endotoxin measurement

As a proxy for early life domestic endotoxin exposure, endotoxin was measured in bedroom floor dust collected when the child was age 12 months. For 47 of the children, because of difficulties associated with scheduling home visits, samples were not available at age 12 months, but bedroom floor dust that had been collected at age 36 months was available and tested. Samples were collected in a 2-m^2 area for 5 minutes onto 70-mm cellulose filters (Whatman International, Maidstone, United Kingdom) with a canister vacuum cleaner (Eureka Mighty Mite, Bloomington, Ind) and a modified collection nozzle (ALK, Horshølm, Denmark). For endotoxin analysis, dust was extracted in pyrogen-free water with 0.05% Tween for 1 hour shaking and centrifuged, and the supernatant was removed and assayed without freezing. Endotoxin was analyzed by the kinetic chromogenic *Limulus* Amebocyte Lysate assay (Cambrex Corp, East Rutherford, NJ), described previously.[18,19] Results are reported both as the concentration of endotoxin per milligram of dust (endotoxin units [EU]/mg) and as the load of endotoxin collected per square meter (EU/m^2).

Dust samples also were collected from various locations in the home and were analyzed for cockroach (Bla g 2, bed, n = 255), mouse (mouse urinary protein [MUP], bed, n = 243), dust mite (Der f 1, bed, n = 209), cat (Fel d 1, bedroom floor, n = 292), and dog (Can f 1, bedroom floor, n = 289) allergens. The procedures for dust collection, allergen extraction, and ELISA have been described previously.[15,20-22]

Questionnaire

After a prenatal questionnaire, a detailed questionnaire including queries about wheezing; runny nose, sneezing, or itchy eyes without a cold (allergic rhinitis symptoms); and report of doctor's diagnosed eczema was administered to the parents about their offspring at ages 12, 24, and 36 months with additional, less detailed health follow-ups at 3, 6, 9, and 30 months. A child was considered to have wheezed if wheeze was reported during at least 1 of the interviews pertaining to that year of life. Other symptoms were recorded similarly.

Statistics

Because of the nonnormal distribution, geometric mean (GM) values (95% CIs) were calculated for endotoxin and means were compared by using ANOVA. Pearson correlations with logarithmically transformed values were used to compare endotoxin and allergen concentrations, and regression analysis was used to evaluate the associations between endotoxin and multiple allergens. Allergen concentrations below the limit of detection were assigned a value of half the limit of detection. Analyses for wheeze and other allergic symptoms were performed by using logistic multiple regression, adjusting for ethnicity, gender, maternal asthma, and smoking in the home. Generalized estimating equations (GEEs) were used to correct variance estimates for analyses of repeated measures. Data were analyzed by using SPSS version 12.0 (Chicago, Ill). GEE was performed by using SAS version 9.0 (Cary, NC).

To test the variability between samples collected at different time points in this cohort, 128 children who had endotoxin measured in a 12-month sample also had endotoxin measured in a 36-month sample. These latter 36-month samples are only included in (1) the correlation between 12-month and 36-month samples from the same home and (2) the analysis for the last sentence of the first health outcome paragraph in the results section.

RESULTS

Study population

Among the 301 mothers, 64% and 36% were of Dominican and African-American ethnicity, respectively, and 19% reported having a doctor diagnosis of asthma. The homes sampled were in East Harlem (18%), the South Bronx (36%), Washington Heights (15%), and West Harlem (32%). Seventy-three percent of the mothers reported an annual household income of $20,000 or less, and 75% reported that they currently received Medicaid at the time the dust sample was collected.

Endotoxin levels

The GM (95% CI) endotoxin concentration in the dust from the bedroom floor samples was 75.9 EU/mg (66-87). Calculated as the endotoxin load per square meter, the GM was 3892 EU/m^2 (3351-4522). There was a significant correlation between endotoxin concentration and load ($r = 0.60$; $P < .001$). There was a modest correlation

TABLE I. GMs of endotoxin concentration (per mg dust) and load (per m^2) by housing and other characteristics

	n For analysis	Study prevalence	GM (95% CI) of endotoxin Concentration (EU/mg)			Load (EU/m^2)		
			No	Yes	P†	No	Yes	P
Carpet in bedroom	295	16%	79 (67-91)	66 (50-87)	.37	3528 (2993-4158)	6757 (4680-9753)	.002
Cat or dog in home	270	13%	77 (65-90)	66 (47-92)	.50	4061 (3408-4840)	3835 (2495-5894)	.82
Leave waste uncovered*	267	15%	76 (64-89)	74 (54-103)	.93	3981 (3323-4771)	4326 (2953-6338)	.73
Eat in bedroom	270	30%	72 (61-86)	83 (64-108)	.39	3969 (3274-4812)	4181 (3088-5662)	.78
Report of mold	269	14%	74 (63-86)	86 (59-127)	.46	4053 (3393-4841)	4036 (2703-6026)	.99
Any housing disrepair‡	271	45%	74 (61-91)	75 (61-93)	.90	4134 (3278-5213)	3923 (3141-4899)	.75
Use wet mop for cleaning	268	56%	93 (73-118)	62 (53-75)	.008	4517 (3498-5835)	3661 (2979-4500)	.21
Medicaid	268	75%	58 (45-73)	80 (68-96)	.047	3144 (2237-4419)	4382 (3651-5259)	.08
Maternal asthma	270	19%	78 (66-92)	65 (49-86)	.34	4011 (3335-4825)	3798 (2741-5261)	.80
Daily cockroach sightings	270	28%	67 (57-80)	99 (77-127)	.02	3599 (2992-4329)	5384 (3891-7449)	.03
Daily mouse sightings	269	16%	69 (59-81)	114 (79-165)	.013	3772 (3167-4491)	5653 (3667-8713)	.07

*Report that they leave their waste uncovered more than once a week.
†ANOVA comparing log-transformed endotoxin values.
‡Report of at least 1 of the following: holes in ceilings or walls, peeling or flaking paint, water damage, leaking pipes, or lack of gas or electricity in the last 6 months.

TABLE II. GM of endotoxin by wheeze, allergic rhinitis symptoms, and eczema, and the adjusted odds ratio (OR) of symptoms associated with endotoxin

	Prevalence of outcome	n For analysis*	GM of endotoxin concentration EU/mg (95% CI)		Adjusted OR†
			No outcome	Yes outcome	
Wheeze 0-12 mo	42%	265	76 (63-93)	73 (58-90)	0.96 (0.78-1.1)
13-24 mo	23%	203	70 (58-86)	102 (76-138)	1.3 (1.01-1.8)
25-36 mo	17%	163	68 (55-83)	70 (48-101)	1.04 (0.71-1.5)
Allergic rhinitis symptoms 0-12 mo	49%	265	69 (55-87)	81 (67-98)	1.1 (0.91-1.4)
13-24 mo	34%	203	79 (64-97)	73 (56-97)	0.98 (0.77-1.3)
25-36 mo	33%	163	66 (53-81)	73 (51-103)	1.08 (0.80-1.5)
Eczema by age 12 mo	22%	265	83 (70-98)	51 (37-71)‡	0.70 (0.53-0.93)
By age 24 mo	27%	203	82 (68-100)	65 (46-90)	0.86 (0.64-1.2)
By age 36 mo	23%	162	74 (60-91)	51 (35-75)	0.74 (0.52-1.05)
			GM of endotoxin load EU/m^2 (95% CI)		
			No outcome	Yes outcome	Adjusted OR†
Wheeze 0-12 mo	42%	265	3667 (2943-4568)	4199 (3292-5356)	1.1 (0.90-1.3)
13-24 mo	23%	203	4093 (3300-5078)	4483 (3198-6284)	1.02 (0.79-1.3)
25-36 mo	17%	163	3954 (3096-5050)	3867 (2987-5006)	1.06 (0.78-1.4)
Allergic rhinitis symptoms 0-12 mo	49%	265	3684 (2856-4751)	4097 (3344-5020)	1.1 (0.89-1.3)
13-24 mo	34%	203	4026 (3196-5072)	4502 (3344-6062)	1.03 (0.82-1.3)
25-36 mo	33%	163	3867 (2987-5006)	4490 (3021-6672)	1.07 (0.84-1.4)
Eczema by age 12 mo	22%	265	3911 (3268-4680)	3779 (2576-5546)	0.95 (0.76-1.2)
By age 24 mo	27%	203	4120 (3339-5082)	4378 (3007-6376)	0.95 (0.73-1.2)
By age 36 mo	23%	162	3864 (3019-4944)	4591 (2923-7210)	1.05 (0.80-1.4)

*Means and OR are taken from the number of subjects used in the logistic regression.
†OR with 95% CI from a logistic regression correcting for sex, maternal asthma, ethnicity, and tobacco smoke exposure in the home.
‡The only univariate significant difference ($P < .05$) in means was observed in endotoxin concentration for eczema at age 0-12 mo ($P = .008$).

between the endotoxin measured at both 12 and 36 months for the same children ($r = 0.18$, $P = .043$; and $r = 0.21$, $P = .019$, for concentration and load, respectively). The associations were stronger when only those children that had not moved between time points (n = 73) were analyzed ($r = 0.26$, $P = .025$; and $r = 0.25$, $P = .035$).

Relationship between endotoxin and allergen levels

We found a modest correlation between endotoxin concentration and mouse allergen (MUP; $r = 0.28$; $P < .001$) and cockroach allergen (Bla g 2; $r = 0.22$; $P <$

.001) concentrations. When both allergens were included as independent variables in a regression model, both Bla g 2 ($\beta = 0.17$; $P = .008$) and MUP ($\beta = 0.24$; $P \leq .001$) were significantly associated with endotoxin ($R^2 = 0.11$ for model). No association was found between the concentration of endotoxin and the concentration of cat allergen (Fel d 1), either when the samples below the limit of detection were included ($r = -0.016$; $P = .79$), or when the analysis was restricted to samples with >1 μg Fel d 1/g (40/292) ($r = -0.19$; $P = .24$). Similarly, we found no association between endotoxin concentration and Can f 1 ($r = -0.013$; $P = .83$) or Der f 1 ($r = 0.089$; $P = .20$).

Associations between cleaning habits, built environment, and endotoxin levels

Higher endotoxin concentrations were associated with frequent sightings of cockroaches and mice, and the reported use of Medicaid (Table I). Lower endotoxin concentration was associated with wet mopping as a home cleaning method. Assuming that wet mopping would be limited to rooms other than the bedroom if a carpet was present, we stratified the analysis by carpet. Endotoxin was only significantly lower in homes that reported wet mopping among those without a carpet in the bedroom ($P = .006$) and not in those that had a carpet ($P = .18$). Higher endotoxin load was associated with a carpet in the bedroom and frequent sightings of cockroaches.

We found no significant differences in either endotoxin concentration or load among cat and/or dog owners compared with nonowners. We also did not find significant associations between endotoxin concentration or load and any of the questions addressing mold or housing disrepair (Table I), or when analyzing using a previously described housing disrepair scale as an independent variable in linear regression ($\beta = 0.02$, $P = .75$ for concentration endotoxin; $\beta = 0.014$, $P = .82$ for load).[23]

Mean endotoxin concentrations were significantly lower ($P = .001$) in East Harlem (57 EU/mg [40-79]) and Washington Heights (53 EU/mg [38-75]) compared with West Harlem (101 EU/mg [78-129]) and the South Bronx (80 EU/mg [65-100]). Endotoxin loads did not differ significantly between these neighborhoods ($P = .19$). Ethnicity is strongly associated with neighborhood; however, there was no difference in either concentration ($P = .48$) or load ($P = .36$) by ethnicity of the mother.

The significant housing characteristics from Table I were evaluated in a linear regression model. For endotoxin concentration, there was an $R = 0.34$ for the model and significant associations for wet mop ($\beta = -0.18$; $P = .004$), East Harlem residence ($\beta = -0.22$; $P = .002$), and Washington Heights residence ($\beta = -0.19$; $P = .008$). For endotoxin load, an $R = 0.29$ was found for the model with significant associations for carpet ($\beta = 0.22$; $P < .001$) and cockroach sightings ($\beta = 0.13$; $P = .035$).

Endotoxin and wheeze, allergic rhinitis symptoms, and eczema

With increasing endotoxin concentration, we found lower adjusted odds of eczema in the first year of life (Table II). In addition, with increasing endotoxin concentration, we found a trend of increasing odds of wheeze during the second year of life. Similar trends (although not significant because of the reduced sample size) were observed when only those children with an endotoxin sample collected at 12 months were examined. No significant associations were detected when the 36-month outcomes were evaluated relative to the 36-month samples only (including those for whom there were also 12-month samples).

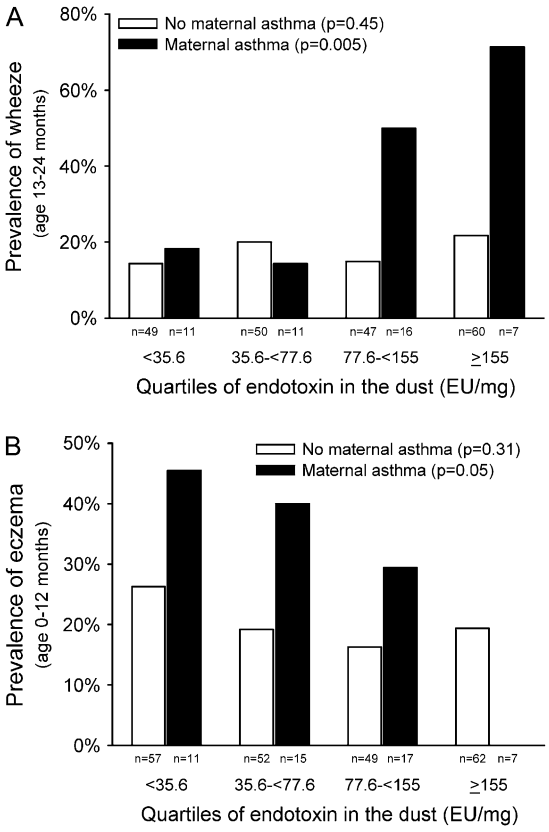

FIG 1. Prevalence of **(A)** wheeze in the 2nd year of life and **(B)** report of physician diagnosed eczema in the 1st year of life by quartile of endotoxin in the bedroom floor dust stratified by maternal history of asthma. *P* values calculated for χ^2 test for trend.

The interaction term *endotoxin by maternal asthma* was significant for wheeze in the 2nd year ($P = .029$) as well as the 1st year ($P = .038$). The interaction term was not statistically significant for eczema in the 1st year ($P = .094$). The prevalence of wheeze and eczema by quartile of endotoxin concentration is shown in Fig 1 stratified by maternal history of asthma.

We also examined the association of endotoxin and wheeze as a longitudinal variable in GEE analysis. Endotoxin concentration was not associated with the presence of wheeze over time ($P = .88$), nor was it associated with change in wheeze over follow-up ($P = .91$). The corresponding *P* values when endotoxin load was evaluated were .91 and .36.

TABLE III. International comparisons of concentration of endotoxin in home dust*

Study location	Dust sampling location	n	Endotoxin (EU/mg)	Associations with endotoxin
Rural New Zealand Wickens et al, 2002[37]	Living room dust from farm homes	94	7.4 GM	Endotoxin↓ on farms ↑Exposure to poultry ↑endotoxin
	Living room dust from nonfarms	188	11.6 GM	No difference with pet No association with allergic disease
Saxony-Anhalt, Germany Gehring et al, 2001[24] Bischof et al, 2002[30]	Living room floor	405	22.7 (1-1200) GM (max-min)	↑Endotoxin ↓sensitization allergens Association strengthened with increasing degree of sensitization
Rural European Community Braun-Fahrlander et al, 2002[4]	Bedding from farm homes	319	37.8 (14.4-88.9) GM (5%-95%)	↑Endotoxin ↓hay fever ↑Endotoxin ↓atopic asthma
	Bedding from nonfarm homes	493	22.8 (8.2-62.9) GM (5%-95%)	↑Endotoxin ↑nonatopic wheeze
US National Survey Thorne et al, 2005[29]	Bedroom floor	588	35.3 (5.0-260) GM (5%-95%)	↑Endotoxin ↑asthma symptoms ↑Endotoxin ↑wheezing
Greater Boston area Park et al, 2001[32] Phipatanakul et al, 2004[25]	Bedroom floor	323	63 (2-761) GM (max-min)	↑Endotoxin ↑wheezing, 1st year life ↑Endotoxin ↓eczema, 1st year life
Northern Manhattan, South Bronx, NYC†	Bedroom floor	301	75.9 (1.2-3388) GM (max-min)	↑Endotoxin ↑wheezing, 2nd year life ↑Endotoxin ↓eczema, 1st year life
Metropolitan Denver Gereda et al, 2001[38] Gereda et al, 2000[3]	Multiple locations	86	178.2 GM	↓Endotoxin for allergen sensitized Pets in home ↑endotoxin Central air-conditioning ↓endotoxin

*Studies selected for comparison included those with endotoxin measurements and allergic or respiratory health outcomes.
†Current study.

DISCUSSION

Inverse associations between endotoxin in house dust and allergic sensitization, eczema, and atopic asthma in children have been reported[3,4,24,25] but have not been evaluated extensively in the inner-city communities of the United States. We have measured endotoxin in the homes of 301 children living in NYC. The novelties of this cohort are that it is limited to an urban inner-city population that is known to have high asthma prevalence and that it includes children with and without maternal asthma. From our findings, we conclude that domestic endotoxin exposure in this community is similar to that in other nonfarming communities, and may be relevant to the development of allergic disease in this population. These similar levels and modest association with health outcomes in turn challenge the notion that lower domestic endotoxin exposure explains the higher asthma prevalence observed in the inner city relative to the suburban US communities.

In comparisons with other studies, endotoxin levels were moderately higher when expressed as a concentration (Table III), but moderately lower when expressed as endotoxin load per square meter (Table IV). We recognize that, with the exception of our comparison with results presented by the US National Survey of Lead and Allergens in Housing that was conducted by the same investigator (P. T.) and laboratory, some caution must be taken when comparing these data with other studies. Variations in the collection and extraction techniques and the *Limulus* assay itself[26,27] can occur. Also, different locations in the home and sampling times may yield different endotoxin levels.[28] In general, we can conclude that endotoxin exposure in these inner-city homes is comparable with that from other studies in which health effects have been associated with domestic endotoxin exposure, but that the load of endotoxin we found is much lower than that from farm homes. Also of interest, these concentrations in the inner city are slightly higher than those reported by the national survey (same laboratory), and this difference is of a similar magnitude to that seen between metropolitan Denver and the greater Boston area (same collection and assay technique).[29]

We identified several housing characteristics associated with higher endotoxin, including reports of cockroach and mouse infestation and their associated measured allergen levels. These findings are in keeping with a German study[30] that found an association with mouse sightings and the US National Home Survey that found an association with cockroach allergen.[29] With regression analysis, mouse and cockroach allergens were found to be

TABLE IV. International comparisons of endotoxin load per square meter in the house dust.

Study location	Dust sampling location	n	Endotoxin (EU/m^2)	Sample collection method
Amsterdam Douwes et al, 2000[39]	Bedroom floor nonsymptomatic	70	2683 (6.7) GM (SD)	1 m^2 for 2 min
	Bedroom floor symptomatic	71	2655 (5.3) GM (SD)	
Northern Manhattan, South Bronx, NYC*	Bedroom floor	301	3892 (35-582,859) GM (max-min)	2 m^2 for 5 min
Rural New Zealand Wickens et al, 2002[37]	Living room dust from farm homes	94	3216 GM	1 m^2 for 1 min
	Living room dust from nonfarms	188	4507 GM	
US National Survey Thorne et al, 2005[29]	Bedroom floor	585	10,470 (1550-113,100) GM (5%-95%)	1.7 m^2 for 5 min
Saxony-Anhalt, Germany Gehring et al, 2001[24]	Living room floor	444	24,221 (160-2,670,001) GM (max-min)	1 m^2 for 2 min
Rural European Community Braun-Fahrlander et al, 2002[4]	Bed dust from farm homes	319	29,897 (5452-157,208) GM (5%-95%)	2 min per m^2
	Bed dust from nonfarm homes	493	14,456 (2915-75,730) GM (5%-95%)	

*Current study.

independently associated with endotoxin; however, these variables combined explained only 11% of the variation in endotoxin. The modest correlation between endotoxin and pests might be driven by pest infestation, which is dependent upon the hygiene of neighboring apartments and other building-wide factors.

This study is the first to report an inverse association between floor dust endotoxin and wet mopping, raising the possibility that this method may be more effective for removing endotoxin-laden dust from its reservoirs in the urban setting. A probable source of endotoxin in inner-city apartments is in the dust tracked into the apartments on shoes. We suspect that those homes that report wet mopping in the apartment also mop the bedroom if there is not a carpet, and this explains the lower levels of endotoxin in the bedroom floor dust specifically among noncarpeted bedrooms. Alternatively, wet mopping could be a proxy for some other housing characteristic not measured in our cohort.

Domestic endotoxin exposure may not be uniform across a large urban environment, as demonstrated by the differences in endotoxin concentrations we measured among the neighborhoods in our study, as well as difference in outdoor airborne endotoxin found by a study from Southern California.[31] In NYC, differences in endotoxin among the neighborhoods may reflect the differences in housing stock. Unlike a German study, we did not find any associations with the story of the apartment.[30] The same German study also reported higher endotoxin in older buildings (before 1980). Although the ages of the buildings were not recorded in this study, we did see a nonsignificant ($P = .07$) trend of increased endotoxin in homes with ceiling heights of 2.7 meters (ie, 9 feet) or higher (GM, 80 EU/mg) compared with those with lower ceilings (60 EU/mg). In NYC, higher ceilings are typically found in older buildings (constructed before World War II).

From the 128 children for whom we had measured endotoxin at both 12 and 36 months, substantial variability in dustborne endotoxin between time points in these homes is evident. In addition, in this urban environment, moving is common, and 20% and 37% of the mothers reported a different address at 24 and 36 months, respectively, than at 12 months. Therefore, the variability in endotoxin within homes and movement to new homes may result in some exposure misclassification, probably biasing the results toward the null and potentially leading to an underestimation of the effect of endotoxin exposure. However, we have used 12-month samples for most of the children (84%), and the 2 significant associations observed (wheeze in year 2, eczema in year 1) represent outcomes within 12 months of the collection.

Two previous studies examined the association between endotoxin and early life asthma/allergy outcomes in urban US populations. Results from a relatively small study in metropolitan Denver found an inverse association with endotoxin concentration and atopy,[3] whereas a positive association between endotoxin concentration and wheeze was found in a mixed urban and suburban birth cohort in Boston.[32,33] This latter Boston study also found an inverse association between endotoxin and eczema.[25]

The novelty of our study comes from the demographics of our population (all urban) and the fact that it was not restricted to children with a family history of asthma or

allergy. Similar to the Boston study, we found both a negative association between endotoxin exposure and eczema and positive association between endotoxin exposure and wheeze.[25,32] The decreased risk for eczema and potential protection from early atopic disease is consistent with the hygiene hypothesis. Given the variety of etiologies (eg, viral infections) that contribute to wheeze in such young children, the positive association between endotoxin and wheeze in the first 2 years does not contradict the hygiene hypothesis. In our study, the association with wheeze was found to be strongest among the children with a maternal history of asthma, which was also reported in a European birth cohort.[34] Some caution must be taken given the relatively small number of children with allergic mothers in our study. Children with a family history of asthma are more likely to develop asthma and wheeze in the first years of life, and this may be an early sign of increased airway hyperreactivity.[35]

Associations with allergic disease (both positive and negative) have been reported with endotoxin expressed both as a concentration and as a load,[3,4,24,32,34] and we have analyzed our results throughout using both measures for comparison. The majority of the home characteristics and the only health effects significantly associated with endotoxin were observed with concentration of endotoxin. The debate over the more valid method persists, with the major critique of concentration misestimation of the actual amount of endotoxin in the sample and the major critique of the load difficulties in standardization of methods between studies.[27,36] Given our findings and those from the Boston cohort, it appears as though analyzing endotoxin expressed as a concentration may be a better method within an urban cohort, possibly because of a methodology less sensitive to collection variation.

In summary, the similarities in endotoxin levels we report (compared with homes elsewhere) and the modest protection from an atopic disease (ie, eczema) and the modest positive association with another (ie, wheeze) lead us to doubt that inner-city homes differ from other nonfarm communities in levels of cleanliness as they pertain to endotoxin exposure. From these findings, we also doubt that low endotoxin exposure explains the higher asthma prevalence observed in inner-city compared with suburban communities in the United States.

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REFERENCES

1. Strachan DP. Hay fever, hygiene, and household size. BMJ 1989;299:1259-60.
2. Strachan DP. Family size, infection and atopy: the first decade of the "hygiene hypothesis. Thorax 2000;55(suppl 1):S2-10.
3. Gereda JE, Leung DY, Thatayatikom A, Streib JE, Price MR, Klinnert MD, et al. Relation between house-dust endotoxin exposure, type 1 T-cell development, and allergen sensitisation in infants at high risk of asthma. Lancet 2000;355:1680-3.
4. Braun-Fahrlander C, Riedler J, Herz U, Eder W, Waser M, Grize L, et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. N Engl J Med 2002;347:869-77.
5. Eisenbarth SC, Piggott DA, Huleatt JW, Visintin I, Herrick CA, Bottomly K. Lipopolysaccharide-enhanced, toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen. J Exp Med 2002;196:1645-51.
6. Martinez FD. The coming-of-age of the hygiene hypothesis. Respir Res 2001;2:129-32.
7. Liu AH. Endotoxin exposure in allergy and asthma: reconciling a paradox. J Allergy Clin Immunol 2002;109:379-92.
8. von Mutius E, Braun-Fahrlander C, Schierl R, Riedler J, Ehlermann S, Maisch S, et al. Exposure to endotoxin or other bacterial components might protect against the development of atopy. Clin Exp Allergy 2000;30:1230-4.
9. Weiss ST. Eat dirt: the hygiene hypothesis and allergic diseases. N Engl J Med 2002;347:930-1.
10. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. Lancet 1998;351:1225-32.
11. Yazdanbakhsh M, Kremsner PG, van Ree R. Allergy, parasites, and the hygiene hypothesis. Science 2002;296:490-4.
12. Asthma facts, 2nd ed. New York: City of New York Department of Health and Mental Hygiene; 2003.
13. Chrischilles E, Ahrens R, Kuehl A, Kelly K, Thorne P, Burmeister L, et al. Asthma prevalence and morbidity among rural Iowa schoolchildren. J Allergy Clin Immunol 2004;113:66-71.
14. Miller RL, Chew GL, Bell CA, Biedermann SA, Aggarwal M, Kinney PL, et al. Prenatal exposure, maternal sensitization, and sensitization in utero to indoor allergens in an inner-city cohort. Am J Respir Crit Care Med 2001;164:995-1001.
15. Chew GL, Perzanowski MS, Miller RL, Correa JC, Hoepner LA, Jusino CM, et al. Distribution and determinants of mouse allergen exposure in low-income New York City apartments. Environ Health Perspect 2003;111:1348-51.
16. Meyer IH, Whyatt RM, Perera FP, Ford JG. Risk for asthma in 1-year-old infants residing in New York City high-risk neighborhoods. J Asthma 2003;40:545-50.
17. Miller RL, Garfinkel R, Horton M, Camann D, Perera FP, Whyatt RM, et al. Polycyclic aromatic hydrocarbons, environmental tobacco smoke, and respiratory symptoms in an inner-city birth cohort. Chest 2004;126:1071-8.
18. Thorne PS. Inhalation toxicology models of endotoxin- and bioaerosol-induced inflammation. Toxicology 2000;152:13-23.
19. Vojta PJ, Friedman W, Marker DA, Clickner R, Rogers JW, Viet SM, et al. First National Survey of Lead and Allergens in Housing: survey design and methods for the allergen and endotoxin components. Environ Health Perspect 2002;110:527-32.
20. Pollart SM, Mullins DE, Vailes LD, Hayden ML, Platts-Mills TA, Sutherland WM, et al. Identification, quantification, purification of cockroach allergens using monoclonal antibodies. J Allergy Clin Immunol 1991;87:511-21.
21. Luczynska CM, Arruda LK, Platts-Mills TA, Miller JD, Lopez M, Chapman MD. A two-site monoclonal antibody ELISA for the quantification of the major Dermatophagoides spp. allergens, Der p I and Der f I. J Immunol Methods 1989;118:227-35.
22. Ingram JM, Sporik R, Rose G, Honsinger R, Chapman MD, Platts-Mills TA. Quantitative assessment of exposure to dog (Can f 1) and cat (Fel d 1) allergens: relation to sensitization and asthma among children living in Los Alamos, New Mexico. J Allergy Clin Immunol 1995;96:449-56.
23. Rauh VA, Chew GL, Garfinkel RS. Deteriorated housing contributes to high cockroach allergen levels in inner-city households. Environ Health Perspect 2002;110(suppl 2):323-7.
24. Gehring U, Bischof W, Fahlbusch B, Wichmann HE, Heinrich J. House dust endotoxin and allergic sensitization in children. Am J Respir Crit Care Med 2002;166:939-44.
25. Phipatanakul W, Celedon JC, Raby BA, Litonjua AA, Milton DK, Sredl D, et al. Endotoxin exposure and eczema in the first year of life. Pediatrics 2004;114:13-8.

26. Douwes J, Versloot P, Hollander A, Heederik D, Doekes G. Influence of various dust sampling and extraction methods on the measurement of airborne endotoxin. Appl Environ Microbiol 1995;61:1763-9.
27. Wickens K, Lane J, Siebers R, Ingham T, Crane J. Comparison of two dust collection methods for reservoir indoor allergens and endotoxin on carpets and mattresses. Indoor Air 2004;14:217-22.
28. Douwes J, Doekes G, Heinrich J, Koch A, Bischof W, Brunekreef B. Endotoxin and beta(1->3) glucan in house dust and the relation with home characteristics: a pilot study in 25 German homes. Indoor Air 1998;8:255-63.
29. Thorne PS, Kulhankova K, Yin M, Cohn R, Arbes SJ Jr, Zeldin DC. Endotoxin exposure is a risk factor for asthma: the National Survey of Endotoxin in U.S. Housing. Am J Respir Crit Care Med 2005;172: 1371-7.
30. Bischof W, Koch A, Gehring U, Fahlbusch B, Wichmann HE, Heinrich J. Predictors of high endotoxin concentrations in the settled dust of German homes. Indoor Air 2002;12:2-9.
31. Mueller-Anneling L, Avol E, Peters JM, Thorne PS. Ambient endotoxin concentrations in PM10 from Southern California. Environ Health Perspect 2004;112:583-8.
32. Park JH, Gold DR, Spiegelman DL, Burge HA, Milton DK. House dust endotoxin and wheeze in the first year of life. Am J Respir Crit Care Med 2001;163:322-8.
33. Litonjua AA, Milton DK, Celedon JC, Ryan L, Weiss ST, Gold DR. A longitudinal analysis of wheezing in young children: the independent effects of early life exposure to house dust endotoxin, allergens, and pets. J Allergy Clin Immunol 2002;110:736-42.
34. Bolte G, Bischof W, Borte M, Lehmann I, Wichmann HE, Heinrich J. Early endotoxin exposure and atopy development in infants: results of a birth cohort study. Clin Exp Allergy 2003;33:770-6.
35. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. N Engl J Med 1995;332:133-8.
36. Platts-Mills TA, Thomas WR, Aalberse RC, Vervloet D, Champman MD. Dust mite allergens and asthma: report of a second international workshop. J Allergy Clin Immunol 1992;89:1046-60.
37. Wickens K, Lane JM, Fitzharris P, Siebers R, Riley G, Douwes J, et al. Farm residence and exposures and the risk of allergic diseases in New Zealand children. Allergy 2002;57:1171-9.
38. Gereda JE, Klinnert MD, Price MR, Leung DY, Liu AH. Metropolitan home living conditions associated with indoor endotoxin levels. J Allergy Clin Immunol 2001;107:790-6.
39. Douwes J, Zuidhof A, Doekes G, van der Zee SC, Wouters I, Boezen MH, et al. (1–>3)-beta-D-glucan and endotoxin in house dust and peak flow variability in children. Am J Respir Crit Care Med 2000;162: 1348-54.